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This deliverable comprises a paper submitted Environmental Toxicology and Chemistry and a paper published in Environmental Science and Technology.

1. Distribution of Polycyclic Aromatic Hydrocarbons in the Food Web of a High Mountain Lake (Pyrenees)
   *Environmental Toxicology and Chemistry*, in press
   **Ingrid Vives, Joan O. Grimalt, Marc Ventura and Jordi Catalan**

2. The roles of food and water in the bioaccumulation of organochlorine compounds in high mountain lake fish.

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   **J. Catalan, M. Ventura, I. Vives and J.O. Grimalt**
DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE FOOD WEB OF A HIGH MOUNTAIN LAKE (PYRENEES)

INGRID VIVES†, JOAN O. GRIMALT*†, MARC VENTURA‡ and JORDI CATALAN‡

†Department of Environmental Chemistry, Institute of Chemical and Environmental Research (CSIC), Jordi Girona 18, 08034-Barcelona, Catalonia, Spain.

*To whom correspondence may be addressed (jgoqam@cid.csic.es)

Disclaimer: The research work reported in this manuscript is new and original. It is not under evaluation for publication in any other journal.
Abstract

The contents of polycyclic aromatic hydrocarbons (PAHs) in the food web organisms included in the diet of brown trout from a remote mountain lake have been investigated. The preferential habitat and trophic level of the component species has been assessed from the signature of stable isotopes ($\delta^{13}$C and $\delta^{15}$N). Subsequently, the patterns of accumulation and transformation of these hydrocarbons in the food chain have been elucidated. Most of the food web organisms exhibit PAH distributions largely dominated by phenanthrene, which agrees with its predominance in atmospheric deposition, water and suspended particles. Total PAH levels are higher in the organisms from the littoral habitat than from the deep sediments or the pelagic water column. However, organisms from deep sediments exhibit higher proportions of higher molecular weight PAH than those in other lake areas. Distinct organisms exhibit specific features in their relative PAH composition that points to different capacities for uptake and metabolic degradation. Brown trout shows an elevated capacity for metabolic degradation since they have lower PAH concentrations than food and they are strongly enriched in lower molecular weight compounds. The PAH levels in trout highly depend on organisms living in the littoral areas. Fish exposure to PAH may therefore vary from lake to lake according to the relative contribution of littoral organisms to their diet.

Keywords: Polycyclic Aromatic Hydrocarbons, Food-web, Trout, Phenanthrene, Invertebrates
INTRODUCTION

The overall environmental concentrations of polycyclic aromatic hydrocarbons (PAHs) have increased extensively in the 20th century as consequence of the general enhancement of combustion processes [1, 2]. These compounds are released directly to the atmosphere, both in the form of gas and in association to particles, where they are transported over long distances and become global contaminants [3]. In this respect, recent studies on sediments, water and air have demonstrated that these compounds are significant pollutants in remote areas such as high mountain lake ecosystems [1, 4-6].

These hydrocarbons and their metabolites have been widely studied because of their carcinogenic and mutagenic properties [7-10]. Thus, some PAH are included in the EU and US lists of priority pollutants [11]. In fish, toxico-hepatic lesions have been related to PAH exposure [12].

One important aspect that needs to be assessed is whether the overall increase of long-range transported of PAH may have an effect on remote ecosystems or in some of their organisms. In this respect, little information is available on the mechanisms of incorporation of these compounds from atmosphere to food web and to high predators such as fish. Whereas fish possess mixed-function oxygenase systems that rapidly metabolize PAH [12-14], these enzymes are poorly developed in some invertebrates that have a lower rate of metabolic degradation [15, 16]. As a consequence, PAHs can be found in benthic and water column organisms even in areas of low pollution [17, 18]. Invertebrates are the main components of the intermediate trophic levels of aquatic food webs [19] and thus their accumulated PAHs are transferred to higher trophic levels, such as fish [13]. Thus, low PAH concentrations in fish do not necessarily imply that they are not receiving significant pollutant fluxes and that they are free of stress. More research is needed to understand to what extent the low degradation
capacity of invertebrates is general and whether PAH mixtures could undergo some degree of transformation. Integrated studies on the PAH pathways throughout the trophic webs up to fish are also required to improve our estimates of fish exposure to those pollutants.

High altitude mountain lakes offer unique environments for the assessment of the transfer mechanisms of atmospherically transported organic pollutants into biota. These lakes do not contain organic contaminant sources in their watersheds. The pollutants found in the organisms originate from atmospheric inputs and are distributed through the complexities of the food web structure and its dynamics. In addition, food webs tend to be simpler than in low land lakes which facilitates sampling and description of the relationships between organisms.

In the present study Lake Redon (Pyrenees) is chosen as model case for these ecosystems. The portion of the food web part related to the diet of the unique top predator in the lake, brown trout (*Salmo trutta*), has been investigated. The study of PAH body contents illustrates how these compounds distribute among the different types of organisms according to their feeding modes and habitats.

**MATERIALS AND METHODS**

**Study area.**

Lake Redon (formerly Redó) (42° 38’ N, 0° 46’ E) is a high mountain lake located in the central Pyrenees (Catalonia, Spain). This lake is situated at 2240 m above sea level, above the regional tree line and far from local pollution sources. It has a surface area of 24 ha, a maximum depth of 73 m and a volume of 7.7 hm³. Water residence time is ca. 4 yr [19] and there is only one outflow. The ice-free period is from June to December [19]. The relative small watershed (155 ha) is scarcely vegetated; alpine meadows alternate with large areas of
Bare granodioritic rock. Lake water composition is very low in phosphorus (total P ca. 0.1 µmol/L) and acid neutralizing capacity (ca. 40 µeq/L) [19].

Sample Collection and Handling.

Fish sampling followed standard test fishing procedures with multifilament gillnets. All fish were measured and dissected and their sex was determined on site. Liver was wrapped in a pre-cleaned aluminium foil and kept frozen (-20ºC) until analysis. Fifteen liver samples were analyzed. The length and weight of the collected specimens were respectively 286 ± 26 mm and 230 ± 58 g (mean ± standard deviation). Average condition factor and age of the trouts analyzed were 0.97 ± 0.09 g·cm⁻³ and 11 ± 4 year old, respectively. There were not significant differences between the analyzed males and females (6 and 7, respectively) on those two factors (Analysis of variance, ANOVA, p < 0.05).

Distinct sampling methods were used for collecting organisms in the three main lake habitats. Kick sampling was chosen for littoral organisms, Ekman drags for deep sediment species and plankton nets for pelagic zooplankton. Samples were kept cold during transport and were later identified and separated in the lab into distinct taxa for stable isotope and PAH analysis. For PAH analyses a minimum wet weight of 0.5 g was obtained pooling individuals of a common taxa. Replicates were analyzed when enough material was available. We tried to include as many organisms as possible from the estimated brown trout diet (Table 1) in the lake. The components finally analyzed were belonging to the littoral habitat, e.g., plecoptera (Arcynopterix compacta, Siphonoperla torrentium), megaloptera (Sialis lutaria), coleoptera (Platambus maculatus), gastropoda (Radix ovata) and cyanobacteria (Nostoc), to the deep sediment habitat, e.g., diptera (chironomidae), crustacea (Eury cercus lamellatus) and bivalvia (Pisidium), and to the pelagic habitat, e.g., crustacea (Daphnia pulicaria).

PAH analysis.
Fish liver was extracted and analyzed for PAHs as described elsewhere [20]. Briefly, liver was ground with activated sodium sulfate spiked with perdeuterated anthracene, pyrene and benzo[ghi]perylene, and introduced in pre-cleaned cellulose cartridges. The tissue was Soxhlet extracted (n-hexane:dichloromethane, 4:1, v/v) for 20 hours and purified through an aluminium oxide chromatographic column. Elution with hexane:dichloromethane (1:2; v/v; 30 mL) contained all the studied PAHs. Further on, extracts were concentrated to 2 mL by vacuum rotary evaporation (20 °C, 20 Torr), then to near dryness under gentle nitrogen flow and redissolved to 50 µl with iso-octane.

Average water content in brown trout tissue (74.2±1.8 %, n=8) was calculated by drying in a vacuum sealed-dissecator at 20ºC to constant weight. This value was used to convert fish wet weight based PAH concentrations in fish to dry weight values for subsequent comparison with invertebrate and algal levels.

These hydrocarbons were analyzed in invertebrates and algae following a method that was slightly modified from the one described above [21]. Briefly, all samples were dried in a vacuum sealed-dissecator at 20ºC to constant weight to determine dry weight. Tissues were Soxhlet extracted with n-hexane-dichloromethane (4:1, v/v) for 20 hours. Then, perdeuterated PAHs were added and clean up was continued as described above.

Before chromatographic analysis, an internal standard of perdeuterated perylene was added to all sample vials as reference to improve injection precision. Samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS, Trace, Thermo, Bremen, Germany). This instrument was equipped with a 50 m x 0.25 mm i.d. HP-5MS capillary column coated with 5% phenyl 95% methylpolysiloxane (film thickness 0.25 µm). Samples were injected in splitless mode. The oven temperature program started at 90ºC (held for 1 min) to 120ºC at 10ºC·min⁻¹, and then to 310ºC at 4ºC·min⁻¹ (holding time 15 min). Injector, transfer line and ion source temperatures were 280ºC, 280ºC and 200ºC, respectively.
Stringent precautions were kept for maintenance of the injector under clean conditions avoiding adsorptions that could deviate the system from linearity and increase the limits of detection and quantification. Helium (1.1 mL·min⁻¹) was used as carrier gas. Data acquisition was in electron impact (70 eV) and selected ion monitoring (40 ms dwell time). The ion mass program is reported elsewhere [4, 22].

All major PAHs were analyzed, including fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene+triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[ah]anthracene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene. Many of them are described as mutagenic, carcinogenic and teratogenic by the International Agency for Research on Cancer [23]. Evidence of oxidation stress in fish from high mountain lakes upon exposure to these compounds has been reported [24].

Procedural blanks were analyzed for every set of six samples. The recoveries of the surrogate standards were calculated for each sample. Average values were 77%, 80% and 120% for perdeuterated anthracene, pyrene and benzo[ghi]perylene, respectively. Identification and quantification of all studied compounds were performed by an external standard method. Relative response to perylene-d₁₀ was calculated and this value was also corrected by the recovery of the surrogate standards.

Residue analysis n-hexane, dichloromethane, iso-octane, methanol, acetone, and analysis grade anhydrous sodium sulfate were from Merck (Darmstadt, Germany). Aluminium foil was rinsed with acetone and let dry at ambient temperature prior to use. Neutral aluminium oxide type 507C was from Fluka AG (Buchs, Switzerland). Cellulose extraction cartridges (20 mm i.d. x 80 mm long) were from Whatman (Maidstone, England). PAHs mix9 and perdeuterated PAHs were purchased from Dr. Ehrenstorfer (Augsburg, Germany).
Aluminium oxide, sodium sulfate and the cellulose cartridges were pre-cleaned by Soxhlet extraction with dichloromethane:methanol (2:1, v/v) for 24 h before use. Sodium sulfate and aluminium oxide were activated overnight at 400°C and 120°C, respectively.

Stable Isotope Analysis

Samples for stable isotope analysis were previously dried during 24 h at 60 °C. In order to assure complete combustion, a small amount of vanadium pentoxide was added before packaging the dried samples in tin capsules. Samples were analyzed in a Delta C Finnigan MAT mass spectrometer (Bremen, Germany) coupled online with a Carlo Erba CHNS (Milan, Italy) elemental analyzer, via a Finnigan conflo 2 interface. Specific standards provided by the International Agency of Atomic Energy (IAEA) were used for calibrating the isotopic signal. Sucrose (IAEA CH6), polyethilene (IAEA CH7) and graphite (IAEA-USGS 24) were used for carbon, and ammonium sulfate (IAEA-USGS 25, IAEA-N1 and IAEA-N2) and potassium nitrate (IAEA-NO3) were used for nitrogen [25]. A complete batch of standards was run at the beginning and at the end of each analytical session, and IAEA CH6 and CH7, and IAEA-N1 and IAEA-NO3 were run every twelve samples for linearity control. Special care was taken in weighting the samples and the standards in order that both had similar amplitudes. Results are reported using atmospheric nitrogen and PeeDee belemnite (PDB) carbonate as references. Reproducibility was better than 0.1 ‰ and 0.3 ‰ for δ13C and δ15N, respectively.

Lipid Content Determination

Lipid content in fish liver was determined gravimetrically. For the rest of organisms we used a different approach because of the low amount of dry mass available. The lipid content was estimated from measured elemental carbon and nitrogen assuming that the main
body constituents were lipids, proteins, ashes and chitin [20, 26, 27]. Lipid percentage was
calculated by difference, after estimating proteins from nitrogen content and multiplying by
6.25 [26], and using literature values for ash [20, 27] and chitin [28] content. This method was
not applicable to Nostoc. Cyanobacteria use carbohydrates as energy reserve, their lipid
content is low and rather constant because it corresponds exclusively to structural compounds.
Therefore, a literature value is used for this organism [29].

RESULTS AND DISCUSSION

Food web structure

The δ\textsuperscript{13}C and δ\textsuperscript{15}N isotopic composition of the samples collected in Estany Redon is
shown in Figure 1. The lowest level of consumers shows a broad differentiation on the δ\textsuperscript{13}C
signature, responding to the δ\textsuperscript{13}C of the food. Values ranged between -29.8‰ and –13.4‰,
the end-members corresponding to small pelagic cladocerans (D. pulicaria) and littoral
invertebrates (R. ovata, P. maculatus), respectively. The organisms living in the deep
sediment habitat showed intermediate δ\textsuperscript{13}C values between -26‰ and -18‰. This range of
values is common when littoral and, particularly, herbivores scraping on benthic algae are
included in the food web studies [25, 31]. Phytoplankton tend to present an elevated isotopic
discrimination respect to the signature of the available water CO\textsubscript{2}, generally showing δ\textsuperscript{13}C
values between –30 to –20‰ [30] but epilithic benthic algae have a lower discrimination, with
values in the range of –20‰ to -8‰ [30] because of the boundary layer effect on diffusive
processes around benthic algae [32]. Sediment matter has intermediate δ\textsuperscript{13}C values between
pelagic and littoral organisms. The characteristic carbon signature in the primary consumers
of the three different habitats is progressively lost at higher trophic levels (Fig. 1), as predators
use food sources from different habitats. Since snails (Radix) present δ\textsuperscript{13}C values of ca. –13‰
we should expect values for epilithic algae (mainly diatoms) of about -12‰ and, in fact, these are the values found in lake Redon in other studies [33].

The lower isotopic values of *Nostoc*, a colony forming cyanobacteria living epilithically, can be interpreted according to two main processes. First, isotopic discrimination is lower in cyanobacterial rubisco than in eukaryotic enzymes. Second, cyanobacteria possess a CO₂ concentrating mechanism that includes active HCO₃⁻ uptake. The bicarbonate contribution to the cyanobacterial δ¹³C signature is higher as lower is the concentration of CO₂ in water [34]. Accordingly, in soft and oligotrophic waters, such are those of lake Redon, low growth rates and scarce free CO₂ result in higher δ¹³C values [35].

The observed δ¹⁵N values ranged between −4.6‰ (*Nostoc* sp.) and 3.6 ‰ (*S. trutta*). Negative δ¹⁵N values for primary producers, which shift the remaining food web values downwards, are found in natural environments or experimental conditions in which nitrogen is in excess with respect to other nutrients required by the algae [36]. This situation is relatively rare in marine systems and terrestrial vegetation but it is common in the present alpine freshwater systems because of the elevated atmospheric deposition of nitrogen pollution [37]. In addition the deposited compounds may have an isotopic signature that is negatively shifted from molecular nitrogen in air [38]. In lake Redon, nitrogen available for algae (NO₃⁻, NH₄⁺) is several orders of magnitude higher than phosphorus [19]. Nitrogen discrimination during algal uptake is therefore expected to be high. Similar or even lower δ¹⁵N values have been found in food webs of alpine streams [39].

Assuming an enrichment factor of 3.5‰ δ¹⁵N per trophic level change [40], the total δ¹⁵N span of Lake Redon involves a short food chain from primary producers to top predators (a mean of 2.2 trophic transferences). Brown trout located at the higher position of the food web do not contain piscivorous specimens. *S. torrentium* and *S. lutaria* belongs to an intermediate level of predators between fish and herbivorous or detritivorous organisms.
Polycyclic aromatic hydrocarbons in the food web

The distributions of PAH in the food web organisms are dominated by phenanthrene (ranging between 22-70 % of the total analyzed PAHs), followed by fluorene, fluoranthene and pyrene (Fig. 2). Anthracene is present in small proportions (between 1-5 % of the total analyzed PAH). The sum of the thirteen PAH considered for study range between 18 (D. pulicaria) and 900 ng g\(^{-1}\) dw (S. torrentium), a 50-fold difference (Table 2). No clear qualitative differences are observed between distinct trophic levels.

Examination of the accumulated amounts exhibits a correspondence with the living habitat of the organisms (Fig. 3). The lowest total value is observed in D. pulicaria, the pelagic species, and the highest values are found in the littoral insect larvae such as P. maculatus, S. lutaria, A. compacta and S. torrentium. In the littoral habitat Nostoc and Radix are the only organisms deviating from these high values. However, they have very low lipid content. After normalization by lipids both organisms appear among those with highest PAH levels (Table 2). The deep sediment organisms, e.g., E. lamellatus, Pisidium and Chironomidae, exhibit intermediate values between littoral and pelagic species.

The higher concentrations in the organisms living in the littoral habitat are in agreement with previous studies in the same lake showing more efficient PAH transfer to underlying sediments at shallower water column. Thus, sediments situated at 72 and 30 m had PAH concentrations of 630 and 2200 ng/g, respectively [41]. These differences suggest that PAH undergo significant degradation upon transport through the water column.

The PAH distributions found in the organisms are predominated by low molecular weight (LMW) compounds, between fluorene and pyrene (Table 2, Fig. 3). Higher relative proportion of high molecular weight (HMW) PAHs are found in the organisms of the deep sediment which constitutes a differential feature from pelagic and littoral species (Fig. 2).
Polycyclic aromatic hydrocarbons in the water column and *D. pulicaria*

Previous studies in Estany Redon have shown that phenanthrene is the dominant PAH in high mountain lake waters, both in the dissolved and the particulate fractions (Fig. 2; [6]). This is also the case in *D. pulicaria*. However, there are a number of compounds that are found in higher proportion in both water phases than in *D. pulicaria*, e.g., fluoranthene, pyrene, chrysene, benzo[ghi]perylene, indicating an apparent selective bioaccumulation of phenanthrene in *Daphnia*. Comparison of the PAH concentrations found in water (dissolved phase) [6] and in *D. pulicaria* (lipid normalized) with the expected octanol-water partitioning ($K_{ow}$) [42] show that phenanthrene and anthracene are higher in the cladoceran than theoretically expected (Fig. 4), which suggest a biomagnification process through particle ingestion. The lack of biomagnification for the other PAH may be due either to selective metabolic degradation, which seems unlikely, or to selective intake during food digestion.

Polycyclic aromatic hydrocarbons in sediments and benthic organisms

The sedimentary PAH composition is dominated by fluoranthene, chrysene, benzo[ghi]perylene and differs significantly from the PAH distributions in the benthic organisms, either those from the littoral zone or in the deep areas. As shown in Figure 2, HMW PAH constitute about 67% of the total sedimentary mixtures of these hydrocarbons whereas the organisms exhibit a higher LMW content with a high predominance of phenanthrene. This later hydrocarbon is the dominant PAH in atmospheric deposition [43], water, suspended particles and most of the organisms. Therefore its decrease in the sedimentary distributions should reflect a preferential degradation with respect to other PAHs.
*E. lamellatus* is the organism exhibiting the highest proportion of HMW PAH and therefore the one with a PAH composition more similar to the one in the sediments (Fig. 2). This cladoceran has a small size that allows it swimming between the sediment debris and eating organic detritus and their associated bacterial flora. The other benthic organisms, including those living partially buried in the sediments such as *Pisidium* and some chironomidae larvae, exhibit a substantial lower amount of HMW PAH than *E. lamellatus.*

In the case of chironomidae, the larvae exhibit the high predominance of phenanthrene that is common to most organisms. However, the pupae are dominated by fluoranthene (Fig. 2). This change in composition occurs during major metabolic changes in the organism because the pupal stage does not involve any feeding. Comparison of the PAH concentrations between larval and pupal stages shows a general PAH decrease in the latter (Table 2). The decrease in fluoranthene is smaller than for the other PAHs, which suggest a lower metabolic degradation rate for this compound in invertebrates. Accordingly, a higher concentration of fluoranthene could be expected as longer is the life time of an invertebrate. This is generally the case of the insects included in the present study (Fig. 2) whose insect larvae are larger and tend to have longer life cycles (*Sialis, Platambus*, plecoptera).

Polycyclic aromatic hydrocarbons in brown trout

Among the distinct fish organs PAH are found in liver and in much lower concentrations in other tissues [8, 44, 45]. This uneven distribution constitutes a major difficulty for food web studies. However, in a first approach, the concentrations in liver can be considered for comparison. The clear predominance of phenanthrene in the trout livers examined in the present study (Fig. 2) is consistent with the PAH profiles found in fish livers of other freshwater [46] and marine systems [22, 47, 48].
For most hydrocarbons, between phenanthrene and benzo[a]pyrene, comparison of the PAH concentrations in liver of *S. trutta* and water with *K*<sub>ow</sub> shows higher ratios than the expected octanol-water partitioning (Fig. 4), suggesting that food and not diffusive exchange through the gills is the likely source of PAH into fish liver. The lower abundance of indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and dibenzo[ah]anthracene may reflect either their higher degree of metabolism degradation or lower fish bioavailability due to their larger size [16].

Comparison between lipid normalized PAHs in the main components of the fish-diet [22] with the PAH content in fish liver (Fig. 5) shows that most of the food organisms accumulate significantly lower concentrations of LMW PAH than *S. trutta*. In contrast, chironomid larvae and *E. lamellatus* accumulate higher concentrations of the HMW PAH than trout. *D. pulicaria* is the only main food trout component whose concentrations do not exceed in any case the amounts found in trout liver.

Calculation of the ratios between lipid normalized PAH concentrations in fish liver and food (Table 1) provides an estimation of the proportion of the PAH intake that is effectively retained in fish (Fig. 5). The PAH values for the pooled fish diet are higher than in fish liver. Having in mind that liver exhibits substantially larger concentrations than the other fish organs, the differences observed indicate that there is a metabolic transformation of PAH in fish which eliminate a substantial part of the ingested hydrocarbons. The selective enrichment of LMW PAH in fish liver when compared to the composition in food intake gives further ground to this observation.

Chironomidae and *D. pulicaria* constitute about 65% of the fish food (Table 1) [33]. The concentrations in these two organisms are lower than in trout liver. Therefore the secondary fish diet components, the organisms from the littoral zones, seem to be very important to explain the PAH levels found in the livers of fish examined in the present study.
This particular habitat may play a key role in the transfer of air transported PAH into high organisms in mountain lakes. Lake Redon is large and deep in comparison to average high mountain lakes. Thus, in this lacustrine ecosystem the difference between central and lateral environments is more significant. The relevance of the organism living in littoral zones for the incorporation of PAH into fish is therefore more apparent. In shallower high mountain lakes that difference may not be so remarkable due to the lower extend of PAH degradation during settling through short water columns.

CONCLUSIONS

Most of the food web organisms exhibit PAH distributions largely dominated by phenanthrene, which contrasts with the PAH composition in sediments, but agrees with its predominance in deposition, water and suspended particles in the water column. PAHs content increase from pelagic to littoral organisms, with intermediate values in organisms inhabiting deep sediments.

In addition to this main pattern, some specific features are related to the distinct organisms or living stages. In the pelagic habitat, *D. pulicaria* shows a relative decrease in HMW compounds with respect to the suspended particles, largely phytoplankton, that constitute its food. *E. lamellatus* shows a PAH distribution that is close to that in sediment whereas other organisms such as *Pisidium* or chironomidae larvae that also live in close contact with the sediment exhibit much higher differences. A significant change in PAH composition is observed between chironomidae larvae and pupae, indicating that metamorphism during the latter stage also involves high metabolic PAH degradation in which fluoranthene is the less affected hydrocarbon. In the littoral habitat, the organisms living
longer also show a higher proportion of fluoranthene. Bioavailability and degradation capacities seem to vary significantly among organisms with different living ways and life cycles.

In fish, the selective enrichment of LMW PAH and the lower lipid normalized PAH concentrations in liver relative to food intake evidences a significant metabolic transformation of these hydrocarbons. Although average fish food shows higher PAH concentration than fish liver, the two main diet components, chironomidae and Daphnia, have lower content. Therefore, the secondary diet components, mainly the organisms from the littoral zone, play a critical role for PAH supply into fish in those mountain lakes. These secondary fish diet components may accumulate higher PAH levels in shallower lakes than the one studied therefore involving higher fish exposure to PAHs.

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REFERENCES


**FIGURE CAPTIONS**

**FIGURE 1.** $\delta^{13}$C and $\delta^{15}$N composition of the organism analyzed for polycyclic aromatic hydrocarbons in this study (Lake Redon, Pyrenees).

**FIGURE 2.** Relative composition of polycyclic aromatic hydrocarbons (%) in the main food web components of Lake Redon (Pyrenees). 1, fluorene; 2, phenanthrene; 3, anthracene; 4, fluoranthene; 5, pyrene; 6, benz[a]anthracene; 7, chrysene+triphenylene; 8, benzo[b]fluoranthene; 9, benzo[k]fluoranthene; 10, benzo[a]pyrene; 11, indeno[1,2,3-cd]pyrene; 12, benzo[ghi]perylene; 13, dibenzo[ah]anthracene.

**FIGURE 3.** Principal component analysis of the content of polycyclic aromatic hydrocarbons (PAH) of the food web organisms from Lake Redon (Pyrenees). The concentrations of abundant compounds in the ordination. The first two principal components accounted for 97.6% of the total variance. Fl, fluorene; Phe, phenanthrene; A, anthracene; Fla, fluoranthene; Py, pyrene; BaA, benz[a]anthracene; Chr, chrysene; BFlas, benzo[b+k]fluoranthenes; BaPy, benzo[a]pyrene; IndPy, indeno[cd-1,2,3]pyrene; BPer, benzo[ghi]perylene; DibahA, dibenzo[ah]anthracene; LMW, PAH between fluorene and pyrene; HMW, PAH between benz[a]anthracene and dibenzo[ah]anthracene. Littoral habitat organisms (squares): Arc, Arcynopterix compacta; Sip, Siphonoperla torrentium, Sia, Sialis lutaria, Pla, Platambus maculatus, Rad, Radix ovata and Nos, Nostoc sp. Deep sediment organisms (diamonds), Chl, Chp, chironomidae larvae and pupae; Eur, Eury cercus lamellatus and Pis, Pisidium. Pelagic organism (circle): Dap, Daphnia pulicaria. Top predator (star): Sal, Salmo trutta.
Comparison between the octanol-water partition coefficients [43] and the ratios between lipid normalized polycyclic aromatic hydrocarbons in *S. trutta* (liver), *D. pulicaria* and water (dissolved phase) [6].

**FIGURE 5.** Representation of the lipid normalized concentrations of polycyclic aromatic hydrocarbons in fish diet, *chironomidae* (larva and pupa), *D. pulicaria* and *E. lamellatus* by reference to the levels in fish liver. The horizontal line gives the reference for compounds in higher or lower lipid normalized concentrations than in fish liver. Numbers in abscissas refer to the PAH list indicated in the caption of Figure 2.
Table 1. Estimated annual averaged brown trout diet in Lake Redon (Pyrenees)

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<td><em>Radix ovata</em></td>
<td>4.7</td>
</tr>
<tr>
<td><em>Platambus maculatus</em></td>
<td>2.5</td>
</tr>
<tr>
<td><em>Pisidium</em> sp.</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Siphonoperla torrentium</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Nostoc</em> sp.</td>
<td>0.2</td>
</tr>
<tr>
<td>Other organisms</td>
<td>6.8</td>
</tr>
<tr>
<td>Unidentifiable material</td>
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</table>
Table 2. Lipid content (%) and concentrations of individual polycyclic aromatic hydrocarbons (PAH) in each species analyzed (ng/g dw). Fl, fluorene; Phe, phenanthrene; A, anthracene; Fla, fluoranthene; Py, pyrene; BaA, benz[a]anthracene; Chr, chrysene; BFlas, benzo[b+k]fluoranthenes; BaPy, benzo[a]pyrene; IndPy, indeno[cd-1,2,3]pyrene; BPer, benzo[ghi]perylene; DibahA, dibenz[ah]anthracene; Low molecular weight (LMW) PAH encompass between fluorene and pyrene; High molecular weight (HMW) PAH encompass between benz[a]anthracene and dibenz[ah]anthracene.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lipid content</th>
<th>FL</th>
<th>Phe</th>
<th>A</th>
<th>Fla</th>
<th>Py</th>
<th>BaA</th>
<th>Chr</th>
<th>BFlas</th>
<th>BaPy</th>
<th>IndPy</th>
<th>BPer</th>
<th>DibahA</th>
<th>total PAH</th>
<th>LMW</th>
<th>HMV</th>
<th>total PAH per lipid</th>
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<td>Littoral habitat</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Nostoc sp.</td>
<td>1.7</td>
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<td>1.8</td>
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<td>0.52</td>
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<td>80</td>
<td>14</td>
<td>43</td>
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<td>Sialis lutaria</td>
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<td>114</td>
<td>16</td>
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<td>7.9</td>
<td>2.9</td>
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<td>7.4</td>
<td>&lt;LOD</td>
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<tr>
<td>Arcynopterix compacta</td>
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<td>92</td>
<td>268</td>
<td>26</td>
<td>117</td>
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<td>11</td>
<td>20</td>
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<td>610</td>
<td>510</td>
<td>98</td>
<td>2299</td>
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<tr>
<td>Siphonoperla torrentium</td>
<td>37.7</td>
<td>117</td>
<td>340</td>
<td>34</td>
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<td>13</td>
<td>74</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
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<td>28</td>
<td>&lt;LOD</td>
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<td>750</td>
<td>150</td>
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<tr>
<td>Eurycercus lamellatus</td>
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<td>92</td>
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<td>55</td>
<td>42</td>
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<td>11</td>
<td>420</td>
<td>230</td>
<td>180</td>
<td>1099</td>
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<tr>
<td>Pisidium sp.</td>
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<td>45</td>
<td>45</td>
<td>4.9</td>
<td>22</td>
<td>13</td>
<td>2.2</td>
<td>12</td>
<td>9.0</td>
<td>1.9</td>
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<td>3.8</td>
<td>&lt;LOD</td>
<td>130</td>
<td>100</td>
<td>34</td>
<td>1012</td>
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<tr>
<td>Chironomidae larvae</td>
<td>30.4</td>
<td>19±6.7</td>
<td>110±21</td>
<td>11±3.2</td>
<td>39±38</td>
<td>24±26</td>
<td>4.3±15</td>
<td>19±41</td>
<td>25±11</td>
<td>7.9±7.8</td>
<td>9.6±16</td>
<td>9.8±9.1</td>
<td>13±10</td>
<td>290±70</td>
<td>200±50</td>
<td>89±21</td>
<td>956</td>
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<tr>
<td>Chironomidae pupae</td>
<td>39.9</td>
<td>10±10</td>
<td>12±7.5</td>
<td>3.0±2.9</td>
<td>36±45</td>
<td>3.9±14</td>
<td>1.2±3.2</td>
<td>4.8±9.6</td>
<td>3.5±3.1</td>
<td>2.2±4.0</td>
<td>2.6±4.8</td>
<td>2.5±3.9</td>
<td>0.24±0.14</td>
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<td>68±35</td>
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<tr>
<td>Pelagic habitat</td>
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<td></td>
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<tr>
<td>Daphnia pulecara</td>
<td>54.4</td>
<td>1.4</td>
<td>10</td>
<td>0.74</td>
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<td>1.1</td>
<td>0.22</td>
<td>0.54</td>
<td>0.72</td>
<td>0.31</td>
<td>0.21</td>
<td>0.28</td>
<td>&lt;LOD</td>
<td>18</td>
<td>16</td>
<td>2.3</td>
<td>33</td>
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<tr>
<td>Top predator</td>
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<td></td>
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<tr>
<td>Salmo trutta</td>
<td>4.6b</td>
<td>9.8±3.4</td>
<td>47±16</td>
<td>3.1±1.9</td>
<td>8.7±5.2</td>
<td>7.5±3.7</td>
<td>0.6±0.6</td>
<td>2.4±1.6</td>
<td>1.5±0.65</td>
<td>0.55±0.60</td>
<td>0.15±0.10</td>
<td>0.20±0.20</td>
<td>0.20±0.15</td>
<td>82±20</td>
<td>76±16</td>
<td>5.6±1.2</td>
<td>1776</td>
</tr>
</tbody>
</table>

a <LOD. Below limit of detection (0.1-0.14 ng/g dw). bLipid content in liver (in muscle 3%).
Figure 1
Figure 2
Figure 3
Figure 4

Conc. organism/mean conc. water

Salmo trutta (liver)  Daphnia pulicaria  kow

Conc. organism/mean conc. water

1.0E+00  1.0E+01  1.0E+02  1.0E+03  1.0E+04  1.0E+05  1.0E+06  1.0E+07  1.0E+08

Phe  A  Fla  Py  BaAnt  Chr  BFlas  BaPy  IndPy  Bper  DibahA
Figure 5

Conc. organism/Conc. fish liver

- Chironomid larvae
- Daphnia pulicaria
- Chironomid pupae
- Eurycercus lamellatus
- fish diet
- Salmo trutta (liver)
An integrated study encompassing the distribution of organochlorine compounds (OC) in water, food web (chironomids, terrestrial insects, cladocers, mollusks, and cyanobacteria), and fish (brown trout) from a high mountain lake (Redon, Pyrenees) is reported. OC distributions in these compartments have been determined to assess their transport routes into fish. Food diets have been estimated by analysis of fish stomach content and food web stable 13C and 15N. OC exchange at fish gill and gut has been evaluated using a fugacity model based on the water, food, and fish concentrations. All compounds exhibited a net gill loss and a net gut uptake. A pseudostationary state was only achieved for compounds with log(Kow) < 6. Calculation of fish average residence times for the compounds in apparent steady state gave values for the compounds in apparent steady state gave values of days to a few weeks for HCHs, 1 year for HCB and 4,4′-DDE, and 2–3 years for 4,4′-DDT and PCB #28 and PCB #52. Residence times longer than one decade were found for the more chlorinated PCB.

Introduction

Chemical pollutants show, in general, higher concentrations at locations closer to the emission sites. However, persistent organic pollutants including some organochlorine compounds (OCs) are found in remote areas without a significant dilution effect. Natural distillation and condensation processes concurrent with atmospheric transport lead to their accumulation in ecosystems and organisms of high latitudes (1–4) or high elevations (5). OCs are mobilized in areas of warm temperatures (ca. mean annual temperature > 5 °C (6)). The more volatile compounds, such as hexachlorobenzene (HCB), hexachlorocyclohexanes (HCH), and low chlorinated polychlorobiphenyls (PCBs), show high accumulation in cold areas located beyond 60° N, with mean annual air temperatures below −5 °C (6). In contrast, the less volatile compounds, such as more chlorinated PCBs (subcooled liquid vapor pressure <10−23 Pa) and DDTs, are also selectively trapped in mountain cold areas (5), which do not reach such low temperatures as the Arctic zone.

Mountain lakes are relatively small in size and very oligotrophic (7), food is scarce, food webs are short, and fish show an opportunistic behavior related to the seasonal availability of food. Unfortunately, the knowledge of the food-web pathways to fish in these environments is scarce (8), and no data on OCs distribution in fish food components are available.

In the present paper we report an assessment of the food pathways to brown trout in a mountain lake using stable isotopes, diet evaluation, and OC content of the food web. Concentrations of OC in food and fish are also compared to the theoretical values expected from their bioconcentration from water levels. Finally, a fugacity model based on the measured OC concentrations in water, food, and fish has been used to evaluate the roles of the gill and gut exchanges. The results are discussed in the context of present knowledge of OC bioaccumulation in fish from high mountain lakes.

Materials and Methods

Study Site. Lake Redon (42°38′N, 0°46′E) is situated at 2240 m above sea level in Central Pyrenees (Catalonia, Spain). It has a surface area of 24 ha, a maximum depth of 73 m, a mean water residence time of about 4 yr, and is usually ice-covered from late December to June (9). The lake is oligotrophic because most of its small watershed (155 ha) is bare rock, and the rest are alpine meadows with scarcely developed soils. The productivity patterns and seasonal changes in the water column are typical for high mountain lakes (7). The lake contains a population of brown trout (Salmo trutta) (10), from which specimens up to 15 years have been collected (11). OC inputs are only related to atmospheric deposition (12). The composition of OC in the waters (13, 14), sediment, and fish (5) of this lake has been described in previous studies.

Sample Collection and Handling. Fish were collected with a series of eight individual bottom gillnets of different mesh sizes (10–46 mm) designed to give the best theoretical catch of brown trout over a range of 10–45 cm. The nets were set perpendicular to the shore at various depths and exposed in the lake for 120 min just at sunrise and sunset. All fish were measured, dissected, and determined for sex on site. Muscle fillets and stomach contents were wrapped in precleaned aluminum foil and kept frozen (−20 °C) until analysis. Brown trout analyzed for OCs (n = 10) averaged (mean ± SD) 265 ± 59 nm in length, 204 ± 118 g in weight, 0.99 ± 0.09 in condition factor, and 7 ± 6 years in age.

A survey of the main food chain lake components was carried out in parallel during the same days of fish sampling. Animals were collected from distinct parts of the lake by kick sampling for littoral organisms, Ekman dredges for sediment species, and plankton nets for zooplankton. Samples were kept cold during transport and were later identified and separated in the lab into distinct classes for stable-isotope and OC analysis.

Brown Trout Diet Evaluation. Gut contents were isolated in the field and kept cold until arrival to the lab where they were then analyzed under a dissecting microscope. The food content was determined mostly up to genus or family level, and the relative percentage was estimated on volume basis.
Organochlorine Compounds Analysis. Fish muscle tissues (5 g wet weight) were extracted and analyzed for OCs as described elsewhere (20). OCs in individuals and Nostoc were determined by grouping for each fish stomach. The degree of taxonomomic resolution was conditioned by the amount of available material for analyses.

<table>
<thead>
<tr>
<th>Operative Food Web Components Analysed for Organochlorine Compounds</th>
<th>Fish</th>
<th>Size (mm)</th>
<th>Age (years)</th>
<th>Foodtype</th>
<th>Feeding Mode</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmo trutta (brown trout)</td>
<td>chordata, fishes</td>
<td>pelagic and littoral</td>
<td>250</td>
<td>7</td>
<td>macroinvertebrates</td>
<td>predator</td>
</tr>
<tr>
<td>Arcynopterix compacta</td>
<td>insecta, plecoptera</td>
<td>benthic</td>
<td>28</td>
<td>2-3</td>
<td>macroinvertebrates</td>
<td>predator</td>
</tr>
<tr>
<td>Siphonoperla torrentium</td>
<td>insecta, plecoptera</td>
<td>benthic</td>
<td>10</td>
<td>1-2</td>
<td>macroinvertebrates</td>
<td>predator</td>
</tr>
<tr>
<td>Sialis lutaria</td>
<td>insecta, megaloptera</td>
<td>benthic</td>
<td>22</td>
<td>2-3</td>
<td>macroinvertebrates</td>
<td>predator</td>
</tr>
<tr>
<td>Polycentropodidae</td>
<td>insecta, trichoptera</td>
<td>benthic</td>
<td>18</td>
<td>1-2</td>
<td>macroinvertebrates</td>
<td>predator</td>
</tr>
<tr>
<td>Platambus maculatus</td>
<td>insecta, coleoptera</td>
<td>benthic</td>
<td>8</td>
<td>2-3</td>
<td>algae, debris</td>
<td>predator</td>
</tr>
<tr>
<td>Chironomidae (larvae)</td>
<td>insecta, diptera</td>
<td>benthic</td>
<td>6</td>
<td>1-2</td>
<td>algae, debris</td>
<td>collector-gathered</td>
</tr>
<tr>
<td>Chironomidae (pupae)</td>
<td>insecta, diptera</td>
<td>pelagic</td>
<td>10</td>
<td>&lt;0.5</td>
<td>none</td>
<td>nonfeeding stage</td>
</tr>
<tr>
<td>Daphnia pulicaria</td>
<td>crustacea, cladocera</td>
<td>planktonic</td>
<td>2</td>
<td>&lt;0.5</td>
<td>phytoplankton, bacteria</td>
<td>collector-filterer</td>
</tr>
<tr>
<td>Eury cercus lamellatus</td>
<td>crustacea, cladocera</td>
<td>littoral</td>
<td>2.5</td>
<td>&lt;1</td>
<td>algae, debris</td>
<td>collector-gathered</td>
</tr>
<tr>
<td>Radix ovala</td>
<td>mollusca, gastropoda</td>
<td>littoral</td>
<td>4</td>
<td>&gt;1</td>
<td>algae, debris</td>
<td>collector-gatherer</td>
</tr>
<tr>
<td>Pisidium sp.</td>
<td>mollusca, bivalvia</td>
<td>littoral</td>
<td>3</td>
<td>&gt;1</td>
<td>phytoplankton</td>
<td>collector-filterer</td>
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<tr>
<td>Nostoc sp.</td>
<td>cyanobacteria</td>
<td>epilithic</td>
<td>5</td>
<td></td>
<td>autotroph</td>
<td>photosynthesis</td>
</tr>
</tbody>
</table>

The degree of taxonomomic resolution was conditioned by the amount of available material for analyses.
TABLE 2. Brown Trout Diet in Lake Redon during Two Distinct Periods of the Ice-Free Season (June and November)*

<table>
<thead>
<tr>
<th>food requirement period</th>
<th>frequency in stomachs (%)</th>
<th>food volume (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>high</td>
<td>low</td>
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<tr>
<td>Chironomidae (pupae)</td>
<td>71.4</td>
<td>0</td>
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<tr>
<td>Daphnia pulicaria</td>
<td>4.8</td>
<td>45.5</td>
</tr>
<tr>
<td>Chironomidae (larvae)</td>
<td>38.1</td>
<td>27.3</td>
</tr>
<tr>
<td>Sialis lutaria</td>
<td>23.8</td>
<td>18.2</td>
</tr>
<tr>
<td>Terrestrial insects</td>
<td>33.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Eury cercus lamellatus</td>
<td>0.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Radix ovata</td>
<td>0.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Polycentropodidae</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Platambus maculatus</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pisidium sp.</td>
<td>29.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Siphonoperla torrentium</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Nostoc sp.</td>
<td>0.0</td>
<td>9.0</td>
</tr>
<tr>
<td>other organisms</td>
<td>33.0</td>
<td>18.0</td>
</tr>
<tr>
<td>unidentifiable material</td>
<td>5.0</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Commonness in the diet is indicated by the frequency that a certain item was found in the stomachs examined (n = 21 and 22, respectively, for high and low food periods). Contribution to the diet is indicated by the percentage of food volume, obtained by weighting percentages of food volume in individual stomachs to the degree of stomach fullness. Annual average contribution to diet was calculated assuming 4 months of high requirements, 4 months of low, and 4 months of very low consumption (stomach fullness - 20%) during the ice covered period with a diet similar to the low food requirement period of the ice-free season. Other reasonable assumptions (i.e., different monthly periods for each type of consumption) do not significantly change the average values.

Results and Discussion

Brown Trout Diet. Sampling was performed in June and November 2000 to cover two extremes of the fish feeding variability during the ice-free period. The availability of some food components (e.g. chironomids) decreases with water temperature as autumn advances. Daily energy (food) requirements also decrease in parallel. These 2 months are therefore representative cases of high and low food demand and availability, respectively. In June, when larvae of aquatic insects were abundant, the average value of the stomach fullness index was 4.4, and no fish with empty stomach was found. In November, during lake overturn and low water fullness, the average value of the index was 1.9, and 9% of the fish had empty stomach. The food items found in the stomachs and their relative contribution were also distinct in the two periods (Table 2). In June, chironomids, either larvae or pupae, were by large the most frequent and abundant. Other organisms, such as terrestrial insects, the bivalve Pisidium, or the megaloptera Sialis, were often found, but their contribution to the food volume was low. In some stomachs chironomid pupae were nearly the sole content, probably because this transient and passive stage facilitates the capture by trout. In November, the more frequent and abundant food components were cladocerans, the pelagic Daphnia, and the littoral Eury cercus. However, they were less dominant that chironomids in June. Other items were also relevant either in abundance or frequency (e.g. chironomids, Radix, Pisidium, Sialis). During this period, the number of unidentifiable items increased, although their contribution to food volume remained very low. Unexpected food items such as colonies of the cyanobacterium Nostoc were also found.

These two snapshots of the trout diet at contrasting periods of the year suggest that these fish mainly feed on chironomids when they are abundant and on alternative prey, particularly small cladocerans such as Daphnia, when they are scarce. The large variety of other food items is complementary.

Isotope Structure of the Food Web. Differences in carbon and nitrogen stable isotope ratios between the distinct food web components provide information on trophic relationships. Commonly used trophic fractionation values are 1½ for δ13C and 3.4½ for δ15N (22, 23), which are similar to mean values found in recent studies of the variation in the trophic fractionation (0.05 ± 0.63½δ13C, 3.49 ± 0.23½δ15N) (24). Due to its lower trophic fractionation, carbon is considered to indicate primary energy sources (e.g. benthic vs pelagic photosynthesis), and nitrogen is used for the discrimination among trophic levels.

The isoto pic signatures of the organisms and primary carbon sources involved in the food web pathways to fish in Lake Redon are shown in Figure 1. The main primary carbon sources—namely littoral (epilithon and Nostoc), pelagic (seston), and organic detritus (sediment)—showed significantly distinct isotopic signatures. The δ13C depletion was larger in seston and deep sediment than in littoral algae, which may reflect the predominant occurrence of primary production in the hypolimnion, below the seasonal thermocline, due to the extreme transparency of the water column (9). Growing temperature in the hypolimnion is significantly lower than in the littoral (ca. 5–10 °C), and available CO2 has a larger contribution from within lake respiration (25). On the other hand, benthic algae tend to be enriched in 13C, due to a boundary layer effect, involving a limitation of CO2 diffusion to the cells that favors the use of bicarbonate as carbon substrate (26).

Fractionation during nitrogen assimilation by algae (phytoplankton and phyto benthos) can be 4 to −5% (27). In our data, pooled epilithon (mainly diatoms) and Nostoc agreed with these values assuming nitrogen sources close to 0% δ15N as expected from its predominant atmospheric origin. However, seston was slightly richer in the heavy isotope than epilithon, pointing to a mixture of phytoplankton and allochthonous matter in the former. Since Daphnia, a planktonic cladoceran mainly feeding on phytoplankton, had a δ15N similar to other herbivores feeding on littoral algae, a common δ15N baseline for the herbivore food web around −4% was assumed.
As expected, brown trout appear to be the unique top predator (Figure 1). However, the average food chain from primary producers to this organism was very short. Assuming an enrichment factor of 3.5 \(\Delta^{15}N\) per trophic level change, the average number of energy transfer steps from primary producers to trout is only 2.2. This is not surprising for a high mountain lake because food availability is scarce due to the oligotrophy of the system, the low inputs from the watershed, and the small lake size (28).

The distribution of the distinct organisms throughout the \(\Delta^{13}C\) gradient indicates a high degree of omnivory. The differences between successive organisms in any trophic chain are significantly lower than 3.5 \(\Delta^{15}N\). Chironomid pupae show higher \(\Delta^{15}N\) values than larvae, although in both cases the species measured were herbivorous. The isotope discrimination could be due to metamorphosis from larvae to pupae. The new form is rebuilt from old tissues, and the transformation may cause an enrichment in \(\Delta^{15}N\) in a similar way as it occurs in starving animals (29) because there is no food intake during the pupae stage. On the other hand, some consumers show a significant contribution of detritus in their diet, particularly the bivalve \(Pisidium\). The \(\Delta^{15}N\) signature of some supposed predators (\(Arcynopterix\), \(Platambus\)) do not agree with an exclusive diet of macroinvertebrates, being closer to scavenger feeding. The narrowing of the \(\Delta^{13}C\) range at increasing \(\Delta^{15}N\) indicates progressive mixing of the pelagic, littoral, and sediment carbon sources at higher food web stages.

The isotopic signatures are consistent with the stomach content observations indicating that trout mainly predate on the herbivore level, constituted by chironomids and cladocerans, with some contribution from other invertebrates. Using the estimated average diet proportions (\(p_i\)) from Table 2, and the measured isotopic signatures of the distinct food items (\(\Delta^{13}C_i, \Delta^{15}N_i\)) the expected isotopic signature for brown trout was calculated as follows:

\[
\Delta^{13}C_{\text{trout}} = \sum p_i \Delta^{13}C_i + 0.05 \quad \text{and} \quad \Delta^{15}N_{\text{trout}} = \sum p_i \Delta^{15}N_i + 3.5
\]

The resulting isotopic values are \(-22.7 \pm 1.8\%o\ \Delta^{13}C\) and \(3.4 \pm 0.80\%o\ \Delta^{15}N\), which are quite similar to the direct fish determinations, \(-22.6 \pm 1.5\%o\ \Delta^{13}C\) and \(3.5 \pm 2\%o\ \Delta^{15}N\). Thus, the above assumptions on average diet composition are feasible and can be considered to provide a good estimate for the annual average composition.

### TABLE 3. \ Organochlorine Concentrations (ng g\(^{-1}\) Dry Weight) in the Most Significant Organisms Involved in the Brown Trout Diet in Lake Redon (Pyrenees)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>lipid content (%)</th>
<th>(\alpha)-HCH</th>
<th>(\gamma)-HCH</th>
<th>HCB</th>
<th>4,4-DDE</th>
<th>4,4-DDT</th>
<th>PCB-28</th>
<th>PCB-52</th>
<th>PCB-101</th>
<th>PCB-118</th>
<th>PCB-133</th>
<th>PCB-138</th>
<th>PCB-180</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmo trutta</em> (muscle)</td>
<td>2.8</td>
<td>0.57</td>
<td>3.54</td>
<td>2.40</td>
<td>57.23</td>
<td>4.19</td>
<td>0.72</td>
<td>1.93</td>
<td>2.82</td>
<td>1.52</td>
<td>9.88</td>
<td>8.52</td>
<td>5.86</td>
</tr>
<tr>
<td>Chironomidae (pupae)</td>
<td>39.9</td>
<td>1.72</td>
<td>12.29</td>
<td>7.18</td>
<td>275.65</td>
<td>27.65</td>
<td>0.93</td>
<td>1.92</td>
<td>6.45</td>
<td>5.36</td>
<td>22.21</td>
<td>14.43</td>
<td>20.50</td>
</tr>
<tr>
<td><em>Daphnia pulicaria</em></td>
<td>54.4</td>
<td>0.03</td>
<td>0.16</td>
<td>0.19</td>
<td>0.11</td>
<td>0.46</td>
<td>0.41</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Chironomidae (larvae)</td>
<td>30.4</td>
<td>0.17</td>
<td>6.05</td>
<td>3.08</td>
<td>58.56</td>
<td>4.98</td>
<td>0.90</td>
<td>1.94</td>
<td>2.33</td>
<td>1.02</td>
<td>4.35</td>
<td>3.32</td>
<td>3.18</td>
</tr>
<tr>
<td><em>Sialis lutaria</em></td>
<td>21.1</td>
<td>0.17</td>
<td>9.38</td>
<td>1.58</td>
<td>14.40</td>
<td>4.75</td>
<td>0.97</td>
<td>0.67</td>
<td>2.38</td>
<td>2.12</td>
<td>6.77</td>
<td>4.92</td>
<td>4.22</td>
</tr>
<tr>
<td><em>Eury cercus lamellatus</em></td>
<td>37.9</td>
<td>0.49</td>
<td>5.19</td>
<td>1.98</td>
<td>60.66</td>
<td>4.34</td>
<td>2.75</td>
<td>1.82</td>
<td>3.07</td>
<td>1.98</td>
<td>2.90</td>
<td>1.64</td>
<td>0.83</td>
</tr>
<tr>
<td><em>Radix ovata</em></td>
<td>0.6</td>
<td>0.12</td>
<td>0.19</td>
<td>0.23</td>
<td>0.00</td>
<td>0.47</td>
<td>0.61</td>
<td>0.08</td>
<td>0.11</td>
<td>0.01</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Polycentropodidae</em></td>
<td>29.8</td>
<td>0.97</td>
<td>6.79</td>
<td>2.65</td>
<td>3.04</td>
<td>1.43</td>
<td>0.46</td>
<td>1.50</td>
<td>2.15</td>
<td>2.27</td>
<td>1.24</td>
<td>1.64</td>
<td>0.51</td>
</tr>
<tr>
<td><em>Platambus maculatus</em></td>
<td>4.9</td>
<td>0.00</td>
<td>14.15</td>
<td>9.24</td>
<td>21.00</td>
<td>4.95</td>
<td>1.76</td>
<td>9.34</td>
<td>8.56</td>
<td>3.18</td>
<td>1.72</td>
<td>1.40</td>
<td>2.35</td>
</tr>
<tr>
<td><em>Pisidium sp.</em></td>
<td>13.2</td>
<td>0.34</td>
<td>1.24</td>
<td>0.87</td>
<td>10.08</td>
<td>1.72</td>
<td>0.73</td>
<td>3.05</td>
<td>2.85</td>
<td>0.97</td>
<td>0.60</td>
<td>0.98</td>
<td>0.13</td>
</tr>
<tr>
<td><em>Siphonoperla torrentium</em></td>
<td>37.7</td>
<td>0.51</td>
<td>19.26</td>
<td>4.60</td>
<td>25.54</td>
<td>12.29</td>
<td>3.60</td>
<td>14.15</td>
<td>9.41</td>
<td>5.63</td>
<td>2.64</td>
<td>3.05</td>
<td>1.24</td>
</tr>
<tr>
<td><em>Nostoc sp.</em></td>
<td>1.7</td>
<td>0.03</td>
<td>0.22</td>
<td>0.08</td>
<td>0.00</td>
<td>0.55</td>
<td>0.25</td>
<td>0.10</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td><em>Arcynopterix compacta</em></td>
<td>26.5</td>
<td>0.99</td>
<td>19.70</td>
<td>3.69</td>
<td>18.17</td>
<td>7.77</td>
<td>4.82</td>
<td>21.72</td>
<td>15.00</td>
<td>7.05</td>
<td>6.77</td>
<td>5.69</td>
<td>4.82</td>
</tr>
</tbody>
</table>

![FIGURE 2. Concentrations of the organochlorine compounds in fish and food-web standardized by lipid content. Horizontal bars indicate the expected values according to the concentrations in water (n = 8) and Kow (31) (values are indicated for high (maximum) and low (minimum) food periods, and mean diet is calculated as described in Table 2).](image-url)
normalized to lipid concentration, brown trout showed the highest levels in all OC. Among the organisms more common in the trout diet, Daphnia showed lower OC concentration values than average, perhaps because of its shorter life span. Combination of the concentrations of the individual organisms to estimate OC content in food shows that the concentrations of these pollutants were slightly lower during the low feeding period (Figure 2). This difference was essentially due to the higher relative contribution of Daphnia to the diet. Mean OC intake was therefore calculated by weighting the lipid normalized concentrations in each food item according to the respective mean contributions to diet (Table 2).

Comparison of the mean OC pooled food concentrations with the theoretical values calculated from OC water content and mean values (30) (Table 4) provides an estimate of the deviation of OC in food web content (normalized to lipids) from thermodynamic equilibrium. A significant number of OCs show concentrations that are close to those expected at equilibrium, namely HCHs, HCB, 4,4′-DDT and PCB congeners #28 and #52 (Figure 2). 4,4′-DDE exhibit values much higher than expected at equilibrium (Figure 2), but 4,4′-DDT shows the opposite trend which could reflect the conversion of 4,4′-DDT into 4,4′-DDE within the organisms (31). In this respect, 4,4′-DDT is always significantly higher than 4,4′-DDE in water (Table 4).

The more chlorinated PCB congeners also deviate from equilibrium since, according to water concentrations, lower values than expected are found. The deviation increases progressively with the degree of chlorination (Figure 2) being larger at higher Kow (Figure 3a). Equilibrium is therefore not reached for OCs with log(Kow) values above ca. 6, indicating that for these OC it could not be reached within the life span (<1 yr) of the organisms more relevant in the trout diet.

In contrast to food, fish show OC concentrations significantly higher than those expected at equilibrium which indicate a biomagnification process (Figure 2) as consequence of food intake. The ratio between the fish and food concentrations, both normalized to lipid content, range between 3.8 for PCB #28 to 16.5 for PCB #138. The ratios between OC in fish and food are also related to Kow (Figure 3b), since the higher is Kow the lower is its loss through the gills. PCB #180 is an exception to this trend (Figure 3b) since it exhibits significant lower biomagnification than expected (Figure 3b). The anomaly could reflect the lower membrane permeation of large molecules (32). In consequence, trout uptake efficiency for PCB #180 seems to be significantly lower than for the other congeners.

The Roles of Food and Water in OC Bioaccumulation.

OC concentration in fish results from the balance between exchanges at the gills during fish respiration, uptake from diet, elimination by fecal egestion, and metabolism and dilution by fish growth. Among these metabolic elimination can be considered of little relevance as compared to the other processes (33).

The relative significance of the other flux components can be evaluated using a fugacity approach (34) in combination with a brown trout food intake model (35) and the measured concentrations in water, food, and fish. For this purpose, a "standard fish" weighting 204 g submitted to the seasonal feeding over year periods divided in three "weather conditions" each involving 4 months with average water temperatures of 8, 4, and 2 °C in an environment of ca. 9 mg O2 L−1 has been taken as lake representative (9). All parts of the fish body were assumed to have the same fugacity. Gill was considered to be a well-mixed compartment in which water flows with oxygen and OCs were transferred inside and outside by diffusion (30). Conductivities (Dw) for gill uptake and loss were equally considered to be dependent from gill ventilation rate (Gw). The net flux in gill exchange (Fg) was determined by the difference between water (fw) and fish (ff) fugacities.

\[
F_g = D_w(f_w - f_f)
\]

The exchanges in the gastrointestinal tract are more complex. Between food uptake and egestion a fraction of matter is removed and there is, in addition, lipid digestion. Therefore, the intestine flux (Fi) required the separate

| TABLE 4. Concentrations of Organochlorine Compounds Dissolved in Water of Lake Redon* |
|----------------------------------------|-----------------|---------------|-------------|-------------|
| pg L⁻¹  | mean b | SD | minimum | maximum |
| α-HCH   | 483    | 225 | 267       | 952       |
| γ-HCH   | 2671   | 1226| 856       | 4846      |
| HCB     | 9.9    | 12  | 0.5       | 35         |
| 4,4′-DDE| 7.4    | 2.3 | 4.1       | 9.7        |
| 4,4′-DDT| 20     | 5.6 | 12        | 28         |
| PCB #28 | 4.3    | 5.3 | 0.4       | 12         |
| PCB #52 | 8.8    | 11  | 1.3       | 33         |
| PCB #101| 6.2    | 5.4 | 1.9       | 17         |
| PCB #118| 3.4    | 2.5 | 1.1       | 8.3        |
| PCB #153| 7.5    | 9.3 | 1.4       | 27         |
| PCB #38 | 8.8    | 9.5 | 1.4       | 25         |
| PCB #180| 4.5    | 5.4 | 0.4       | 14         |

* Data from refs 13 and 14 and unpublished. b Average values of data collected in July 96 at 1, 5, and 60 m depth; June 97 at 1 m, 5, 25, and 59 m depth (n = 4) and November 00 at 1 m depth (n = 8).
consideration of uptake (DA) and loss (DE) conductivities
\[ F_i = D_A(f_A - f_E f) \]  
where \( f_A \) is food fugacity.

\( D_A \) is commonly modeled to depend on food consumption rate (GA) and gut absorption efficiency (EA) (20, 33, 36). \( D_E \) was taken proportional to GA (1-\( \beta \)), where \( \beta \) was the fraction of ingested diet absorbed by the organism. Development of the two equations according to the definitions in Table 5 provided the following expressions for the two fluxes:

\[ F_g = G_w(C_w - C_f(L_f K_{ow})) \]  
\[ F_i = G_A(C_A E_A - C_f(1-\beta)) \]

Apart from water and food concentrations, the relative flux differences result from the values of the \( K_{ow} \) and \( E_A \) coefficients, the latter being particularly relevant for differentiating the behavior of PCB #180. Gw and GA determine the absolute flux values. The two rates depend on the fish daily energy requirements (37), which under optimal conditions result from the body weight and temperature in a nonlinear way. \( G_A \) was estimated using Elliot’s model for brown trout (35) and distinguishing the three feeding periods mentioned above. \( G_w \), in addition to oxygen consumption as determined by the energy requirement, was made dependent on the oxygen in the water and uptake efficiency (36) (Table 5).

The resulting calculations show that a number of OCs were close to a steady state, namely HCB, 4,4′-DDE, PCB #28, and PCB #52 (Figure 4). These are the compounds that, in turn, are also in equilibrium between water and food. This parallelism gives ground to the assumptions for the calculations of exchanges in the gills and intestine, at least in relative terms. Since HCHs and 4,4′-DDT have also log(\( K_{ow} \)) < 6, steady state for these OC should be expected, but this is not the case. In 4,4′-DDT gut uptake appears to be higher than gill net loss, which may reflect a metabolic transformation within the fish. In HCHs, gill loss is much higher than gut uptake, which is unlikely unless the assumed water fugacity does not correspond to the one presently experienced by fish. This can certainly be the case as the calculations are based on long time water concentrations averages, and fish renewal time of the more volatile compounds is a matter of days.

**TABLE 5. Definition of Symbols and Summary of the Parameters Used in the OC Fish Flux Calculations**

<table>
<thead>
<tr>
<th>parameter</th>
<th>units</th>
<th>definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_W, f_A, f_F )</td>
<td>Pa</td>
<td>water, food and fish fugacities, ( f = C_i Z^{-1} ) concentration</td>
</tr>
<tr>
<td>( C_i )</td>
<td>pg L(^{-1} )</td>
<td>fugacity capacity, ( Z = L_i K_{ow} H^{-1} ) octanol-water partition coefficient</td>
</tr>
<tr>
<td>( Z_i )</td>
<td>L(^{-1} ) Pa(^{-1} )</td>
<td>Henry’s law constant</td>
</tr>
<tr>
<td>( K_{ow} )</td>
<td>L kg(^{-1} )</td>
<td>conductivity at gills, ( G_w = G_w Z_w ) gill ventilation rate, ( G_w = K_{ow} O_{2w}^{-1} E_{ox} ) fish daily intake requirement, ( K = f(T, W) ) temperature</td>
</tr>
<tr>
<td>( H )</td>
<td>Pa L pg(^{-1} )</td>
<td>fish weight</td>
</tr>
<tr>
<td>( D_w )</td>
<td>pg d(^{-1} ) Pa(^{-1} )</td>
<td>energy to oxygen consumption coeff, 0.047 water oxygen concentration</td>
</tr>
<tr>
<td>( G_w )</td>
<td>L d(^{-1} )</td>
<td>efficiency of oxygen uptake, 0.45 gut uptake conductivity, ( D_a = E_a G_A Z_a ) food consumption rate, ( G_a = K_{ef} ) energy to food volume consumption coeff, ( 10^{-6} ) gut uptake efficiency, 0.75 (except for PCB #180, 0.45)</td>
</tr>
<tr>
<td>( D_A )</td>
<td>pg d(^{-1} ) Pa(^{-1} )</td>
<td>gut loss conductivity, ( D_a = G_a (1-\beta) Z_a ) fraction of ingested diet absorbed by the fish, 0.8</td>
</tr>
<tr>
<td>( \beta )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 4.** Comparison of the calculated net gill loss and net gut uptake of organochlorine compounds in a fish of 204 g in Lake Redon according to the measured concentrations in water, food, and fish.

An average residence time in fish can be calculated for the compounds in apparent steady state. For HCHs it is in the order of some days to a few weeks, for HCB and 4,4′-DDT it is about 1 year, and for 4,4′-DDT, PCB #28, and #52 it is about 2 or 3 years. For the rest of the compounds steady state is not achieved, but the present turnover indicates characteristic times around a decade for PCB #101 and two or three decades for PCBs #110 to #153. In the case of PCB #180, a fish could hardly achieve a steady state at present exposures unless it lived for centuries.

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